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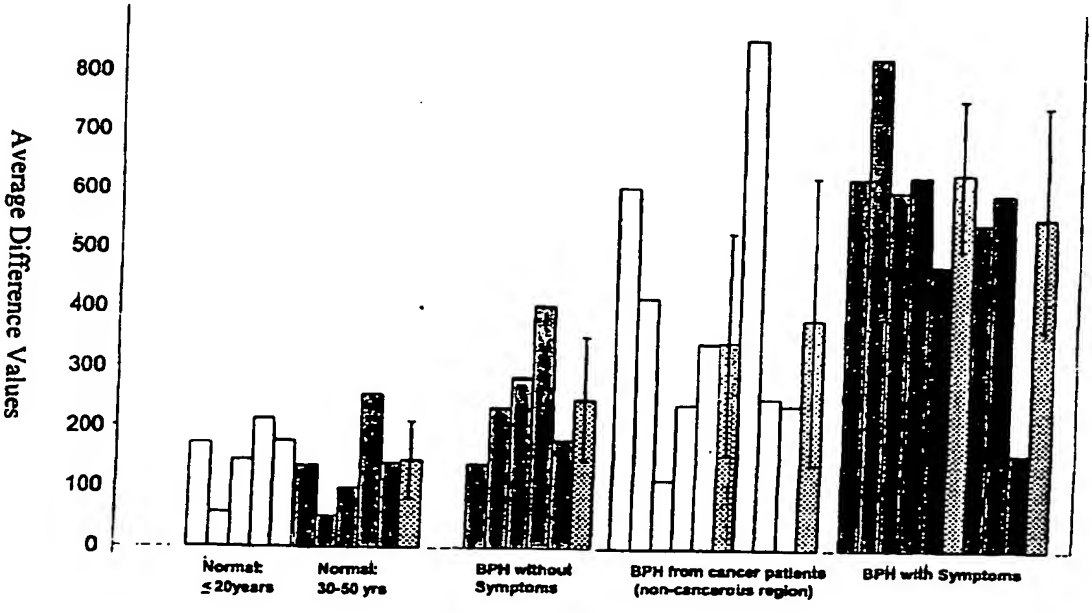

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(57) Abstract: The invention relates generally to the changes in gene expression in Benign Prostatic Hyperplasis (BPH). The invention relates specifically to the human gene Del-1 which is differentially expressed in BPH compared to normal prostate tissue.

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DEL-1 AND BENIGN PROSTATIC HYPERPLASIA

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RELATED APPLICATION

This application is related to U.S. Provisional Application 60/245,674, filed November 6, 2000 which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The invention relates generally to the changes in gene expression in prostate tissue removed from male patients with benign prostatic hyperplasia (BPH). The invention specifically relates to a human gene which is differentially expressed in BPH tissue compared to normal prostate tissue.

BACKGROUND OF THE INVENTION

Benign Prostatic Hyperplasia (BPH)

BPH is the most common benign tumor in men over the age of 60. It is estimated that one in four men living to the age of 80 will require treatment for this disease. BPH is usually noted clinically after the age of 50, the incidence increasing with age, but as many as two thirds of men between the ages of 40 and 49 demonstrate histological evidence of the disease.

The anatomic location of the prostate at the bladder neck enveloping the urethra plays an important role in the pathology of BPH, including bladder outlet obstruction. Two prostate components are thought to play a role in bladder outlet obstruction. The first is the relatively increased prostate tissue mass. The second component is the prostatic smooth muscle tone.

The causative factors of BPH in man have been intensively studied (see Ziada *et al.*, *Urology* 53:1-6, 1999). In general, the two most important factors appear to be aging and the presence of functional testes. Although these factors appear to be key to the development of BPH, both appear to be nonspecific.

Del-1 protein and gene

A protein known as integrin-binding protein or Developmentally-regulated Endothelial cell Locus 1 protein (Del-1 or Del1) has been characterized as a matrix protein

having 3 epidermal growth factor (EGF)-like domains and 2 discoidin I / Factor VIII like domains. An RGD motif in the B-loop of the second EGF-like repeat is the likely site of interactions between Del-1 and integrin receptors, primarily αvβ3. This matrix protein may have an autocrine function as it shows an endothelial cell-specific expression pattern and triggers αvβ3-dependent cellular responses typical of these vascular cells. Through integrin-mediated signaling via the Ras-mitogen activated protein kinase (MAPK) pathway, Del-1 promotes endothelial cell adhesion, migration and can elicit either angiogenic and anti-angiogenic cellular responses under various experimental circumstances. Such activities support a role for Del-1 in complex processes of angiogenesis and vascular remodeling (Hidai *C et al., Genes & Development* 12:21-33, 1998 and Penta K *et al., J Biol Chem* 274:11101-9, 1999).

The Del-1 protein and gene have been described in US patent 5,874,562, US patent 5,877,281 and WO patent publication 96/40769. GenBank accession numbers for various forms of human and murine Del-1 are U70312 (human), U70313 (human, Z20 splice variant), AF31524 (major murine form) and AF 31525 (minor murine form, amino terminal truncation due to alternate splice site) all of which are herein incorporated by reference.

Molecular Changes in BPH

Little is known about the molecular changes in prostate cells associated with the development and progression of BPH. It has been demonstrated that the expression levels of a number of individual genes are changed compared to normal prostate cells. These changes in gene expression include a decreased level of Wilm's tumor gene (WT-1) and increased expression of insulin growth factor II (IGF-II) (Dong *et al., J Clin Endocrin Metab* 82:2198-2203, 1997).

While the changes in the expression levels of a number of individual genes have been identified, the investigation of the global changes in gene expression has not been reported. Accordingly, there exists a need for the investigation of the changes in global gene expression levels as well as the need for the identification of new molecular markers associated with the development and progression of BPH. Furthermore, if intervention is expected to be successful in halting or slowing down BPH, means of accurately assessing the early manifestations of BPH need to be established. One way to accurately assess the early manifestations of BPH is to identify markers which are uniquely associated with disease progression. Likewise, the development of therapeutics to prevent or stop the progression of BPH relies on the identification of genes responsible for BPH growth and

function.

SUMMARY OF THE INVENTION

The present invention is based on the discovery that the Del-1 gene is differentially expressed in BPH tissue compared to normal prostate tissue. The invention provides methods of diagnosing BPH in a patient comprising: acquiring a tissue, blood, urine or other sample from a subject; and determining the level of expression of a polypeptide or a nucleic acid molecule encoding Del-1 or a variant thereof. The invention further provides methods of diagnosing BPH wherein the Del-1 protein or variant thereof is encoded by (a) an isolated nucleic acid molecule that encodes the amino acid sequence of SEQ ID NO: 2; or (b) an isolated nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule that encodes the amino acid sequence of SEQ ID NO: 2.

The invention further provides methods of identifying binding partners for a Del-1 protein or variant thereof, comprising: exposing Del-1 to a potential binding partner derived from prostate cells or tissue; and determining if the potential binding partner binds to Del-1, thereby identifying binding partners for the Del-1 protein or variant thereof. The cells of this method may be *in vitro*, may be obtained from human biopsy samples, or may be obtained from an animal model of prostate disease.

The invention further provides methods of identifying an agent which modulates the expression of a nucleic acid encoding a Del-1 protein or variant thereof, comprising: exposing prostate cells to the agent; and determining whether the agent modulates expression of said nucleic acid, thereby identifying an agent which modulates the expression of a nucleic acid encoding the Del-1 protein or variant thereof. The cells of this method may be *in vitro*, may be obtained from human biopsy samples, or may be obtained from an animal model of prostate disease.

The invention further provides methods of identifying an agent which modulates at least one activity of a Del-1 protein or variant thereof, comprising: exposing prostate cells to the agent; and determining whether the agent modulates at least one activity of the Del-1 protein or variant thereof, thereby identifying an agent which modulates at least one activity of the Del-1 protein or variant thereof. The cells of this method may be *in vitro*, may be obtained from human biopsy samples, or may be obtained from an animal model of prostate disease.

The invention further provides methods of modulating the expression of a nucleic

acid encoding a Del-1 protein or variant thereof, in a subject with benign prostatic hyperplasia, comprising: administering to the subject an effective amount of an agent which modulates the expression of a nucleic acid encoding the Del-1protein or variant thereof.

The invention further provides methods of modulating at least one activity of a Del-1 protein or variant thereof, in a subject with benign prostatic hyperplasia, comprising: administering to the subject an effective amount of an agent which modulates at least one activity of a Del-1 protein or variant thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Figure 1 shows the expression of Del-1 (AA256268) in prostate biopsy samples from normal human patients (aged ≤ 20 years or 30-50 years) and BPH patients, including BPH samples from individuals without symptoms, samples from BPH patients without symptoms who were diagnosed with prostate cancer, and samples from BPH patients with symptoms. In all cases, the subregion of the prostate analyzed was the transitional zone. The group average standard deviation is shown to the right of individual values within each disease group, and is also shown in Figure 3 for comparison with other normal tissues.

Figure 2 Figure 2 shows the tissue distribution of RNA encoding the protein of Del-1 (about 5.0 kb) as analyzed by Northern blot in human heart, brain, spleen, lung, liver, smooth muscle, kidney and testes (lanes 1-8, respectively). Lane M contains RNA markers, indicated by small dots (4.4 and 7.5 kb markers are labeled).

Figure 3 Figure 3 shows an electronic Northern, in which the expression level of AA256268 was measured across a panel of normal tissues and tissues from subtypes of BPH patients (see Figure 1) using the Affymetrix human Hu35KsubA GeneChip set. For each tissue type, the mean SDM is shown as a horizontal bar graph for samples obtained from 3 or more normal individuals.

Figure 4 Figure 4 shows the results of semi-quantitative PCR for the expression of AA256268 in various tissues. Upper panel of the gel: 25 cycles. Lower gel panel: 30 cycles. Double lanes are shown for each tissue: the primer pair F156-177/R759-770 were used for the first lane per tissue, and primers F182-201/R780-801 were used for the second lane per tissue. Lanes: M) 100 bp ladder; 1) heart; 2) brain; 3) leukocytes; 4) lung; 5) liver; 6) fetal brain; 7) kidney; 8) spleen; 9) placenta; 10) BRF 55; 11) glomeruli; and 12) osteoblast.

Figure 5 Figure 5 is a hydrophobicity plot (PEPPLOT) of AA256268. Analysis was done using the methods of Goldman *et al.* and of Kyte-Doolittle.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

I. General Description

The present invention is based in part on the identification of a gene family that is differentially expressed in human BPH tissue compared to normal human prostate tissue. This gene family corresponds to a human gene that encodes a protein of 480 amino acids (Del-1 or AA256268). The gene that encodes this human protein is also present in mice, and may be found in other animal species, particularly mammalian species.

The Del-1 protein or variants thereof can serve as a targets for agents that modulate gene expression or activity in prostate cells. For example, agents may be identified that modulate biological processes associated with prostate growth, including the hyperplastic process of BPH.

The present invention is further based on the development of methods for isolating binding partners that bind to the Del-1 protein or variants thereof in prostate cells. Additionally, the Del-1 protein or variants thereof provide novel targets for the screening of synthetic small molecules and combinatorial or naturally occurring compound libraries to discover novel therapeutics to regulate prostate function.

II. Specific Embodiments

A. The Protein Associated with BPH

The methods of the present invention measure the level of expression of a nucleic acid molecule encoding a Del-1 protein or a variant thereof. As used herein, "Del-1" refers, in part, to the human amino acid sequence depicted in SEQ ID NO: 2, and "protein" or "polypeptide" refers, in part, to a protein that has the human amino acid sequence depicted in SEQ ID NO: 2. The term "variant" in the phrase "a Del-1 protein or a variant thereof" refers to any naturally occurring allelic variant, splicing variant or protein that has a slightly different amino acid sequence than that specifically recited above. Allelic variants, though possessing a slightly different amino acid sequence than that recited above, will still have the same or similar biological functions associated with the Del-1 protein.

The variants of the Del-1 protein are further defined to include insertion, deletion or amino acid substitution variants of the sequence set forth in SEQ ID NO: 2. Insertion

variants are those in which one or more amino acid residues has been inserted N- or C-terminal to, or within, the disclosed sequence. The substitution, insertion or deletion of one or more amino acids may or may not be a conservative variation. As used herein, a substitution, insertion or deletion is conservative if the alterations in the amino acid sequence do not adversely affect the biological functions of the protein. A substitution, insertion or deletion is said to adversely affect the protein when the altered sequence prevents or disrupts a biological function associated with the protein. For example, the overall charge, structure or hydrophobic/hydrophilic properties of the protein can be altered without adversely affecting a biological activity. Accordingly, the amino acid sequence can be altered, for example to render the peptide more hydrophobic or hydrophilic, without adversely affecting the biological activities of the protein.

Ordinarily, the allelic variants, the conservative substitution variants, and the members of the protein family, will have an amino acid sequence having at least about 50%, 60%, 70% or 75% amino acid sequence identity with the sequence set forth in SEQ ID NO: 2, more preferably at least about 80%, even more preferably at least about 90%, and most preferably at least about 95% sequence identity. Identity or homology with respect to such sequences is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the known peptides, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity (see section B for the relevant parameters). Fusion proteins, or N-terminal, C-terminal or internal extensions, deletions, or insertions into the peptide sequence shall not be construed as affecting homology.

The Del-1 protein variants also include fragments of the protein disclosed in SEQ ID NO: 2, and the related variants discussed herein, having a consecutive sequence of at least about 3, 4, 5, 6, 10, 15, 20, 25, 30, 35 or more amino acid residues of these proteins; amino acid sequence variants wherein one or more amino acid residues has been inserted N- or C-terminal to, or within, the disclosed coding sequence; and amino acid sequence variants of the disclosed sequence, or their fragments as defined above, that have been substituted by another residue. Such fragments, also referred to as peptides or polypeptides, may contain antigenic regions, functional regions of the protein identified as regions of the amino acid sequence which correspond to known protein domains, as well as regions of pronounced hydrophilicity. The regions are all easily identifiable by using commonly available protein sequence analysis software such as MacVector

(Oxford Molecular).

As used herein, the family of proteins and variants related to the human amino acid sequence of SEQ ID NO: 2 also refers to proteins that have been isolated from organisms in addition to humans. The methods used to identify and isolate other members of the family of proteins related to these proteins are sell known (see Sambrook *et al.*, Molecular Cloning - A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Contemplated variants further include those containing predetermined mutations by, *e.g.*, homologous recombination, site-directed or PCR mutagenesis, and the corresponding proteins of other animal species, including but not limited to rabbit, mouse, rat, porcine, bovine, ovine, equine and non-human primate species, and the alleles or other naturally occurring variants of the family of proteins; and derivatives wherein the protein has been covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid (for example a detectable moiety such as an enzyme or radioisotope).

The level of expression of members of the family of Del-1 proteins can be measured in terms of the nucleotide product(s) of transcription, for example including but not limited to total RNA or mRNA, or in terms of the protein product(s) of translation, including but not limited to the protein at any stage before or after one or more post-translational cellular events including but not limited to, folding, cleavage, glycosylation and phosphorylation, binding (e.g. to a receptor or an antibody), labeling, purification or isolation.

As described below, members of the family of proteins can be used: (1) to identify agents which modulate at least one activity of the protein; (2) to identify binding partners for the protein, and (3) as a therapeutic agent or target.

B. Nucleic Acid Molecules

The methods of the present invention measure the level of expression of a nucleic acid molecule that encodes the protein having SEQ ID NO: 2 and the related protein variants herein described. As used herein, "nucleic acid" is defined as RNA or DNA that encodes a protein or peptide as defined above, or that hybridizes to the complement of a nucleic acid that encodes the amino acid sequence of SEQ ID NO: 2 and remains stably bound to it under appropriate stringency conditions, encodes a polypeptide sharing at least about 50%, 60%, 70% or 75% sequence identity, preferably at least about 80%,

more preferably at least about 85%, and even more preferably at least about 90% or 95% or more identity with the Del-1 peptide sequence or variants thereof, or exhibits at least about 50%, 60%, 70%, 80%, 90% or 95% or more nucleotide sequence identity over the open reading frame of SEQ ID NO: 1. Specifically contemplated are RNA molecules such as total RNA and mRNA.

Homology or identity at the nucleotide or amino acid sequence level is determined by BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin *et al., Proc Natl Acad Sci USA* 87:2264-2268, 1990 and Altschul, J Mol Evol 36:290-300, 1993, fully incorporated by reference) which are tailored for sequence similarity searching. The approach used by the BLAST program is to first consider similar segments between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al., Nature Genetics* 6:119-129, 1994, which is fully incorporated by reference. The search parameters for histogram, descriptions, alignments, expect (*i.e.*, the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al., Proc Natl Acad Sci USA* 89:10915-10919, 1992, fully incorporated by reference). For blastn, the scoring matrix is set by the ratios of M (*i.e.*, the reward score for a pair of matching residues) to N (*i.e.*, the penalty score for mismatching residues), wherein the default values for M and N are 5 and -4, respectively. Four blastn parameters were adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every winkth position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent Blastp parameter settings were Q=9; R=2; wink=1; and gapw=32. A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

"Stringent conditions" are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C., or (2) employ during hybridization a denaturing agent such as formamide,

for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is hybridization in 50% formamide, 5× SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5× Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2× SSC and 0.1% SDS. A skilled artisan can readily determine and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal. Preferred molecules are those that hybridize under the above conditions to the complement of SEQ ID NO: 1 and which encode a functional protein. Even more preferred hybridizing molecules are those that hybridize under the above conditions to the complement strand of the open reading frame of SEQ ID NO: 1.

A nucleic acid molecule encoding a Del-1 protein, or a variant as described above, includes fragments of the encoding nucleic acid molecule. As used herein, a fragment of an encoding nucleic acid molecule refers to a small portion of the entire protein coding sequence.

The size of the fragment will be determined by the intended use. For example, if the fragment is chosen so as to encode an active portion of the protein, the fragment will need to be large enough to encode the functional region(s) of the protein. For instance, fragments which encode peptides corresponding to predicted antigenic regions may be prepared (see Figure 5).

If the fragment is to be used as a nucleic acid probe or PCR primer, then the fragment length is chosen so as to obtain a relatively small number of false positives during probing/priming (see the discussion in Section G).

Fragments of the nucleic acid molecules encoding a Del-1 protein or a variant thereof, (*i.e.*, synthetic oligonucleotides) that are used as probes or specific primers for the polymerase chain reaction (PCR), or to synthesize gene sequences encoding Del-1 protein or a variant thereof, can easily be synthesized by chemical techniques, for example, the phosphoramidite method of Matteucci *et al.* (*J Am Chem Soc* 103:3185-3191, 1981) or using automated synthesis methods. In addition, larger DNA segments can readily be prepared by well known methods, such as synthesis of a group of oligonucleotides that define various modular segments of the gene, followed by ligation of oligonucleotides to build the complete modified gene.

The encoding nucleic acid molecules of the present invention may further be

modified so as to contain a detectable label for diagnostic and probe purposes. A variety of such labels are known in the art and can readily be employed with the encoding molecules herein described. Suitable labels include, but are not limited to, biotin, radiolabeled nucleotides and the like. A skilled artisan can readily employ any such label to obtain labeled variants of the nucleic acid molecules encoding a Del-1 protein or a variant as described above.

C. Methods to Identify Binding Partners

Another embodiment of the present invention provides methods for isolating and identifying binding partners of Del-1 protein or a variant thereof. In general, a Del-1 protein or a Del-1 variant is mixed with a potential binding partner in an extract of a cell under conditions that allow the association of potential binding partners with the protein. After mixing, peptides, polypeptides, proteins or other molecules that have become associated with a Del-1 protein or variant of the invention are separated from the mixture. The binding partner that bound to the protein of the invention can then be removed and further analyzed. To identify and isolate a binding partner, the entire Del-1 protein, for instance a protein comprising the entire amino acid sequence of SEQ ID NO: 2, can be used. Alternatively, a fragment of the protein can be used.

As used herein, a cellular extract refers to a preparation or fraction which is made from a lysed or disrupted prostate cell. The preferred source of cellular extracts will be cells derived from human prostate tissue or cells, for instance, biopsy tissue or tissue culture cells from BPH patients. Animal models of prostate disease, as are known to persons of ordinary skill in the art, are another source of cells for cellular extract. For example, see Coffey DS and Walsh PC, "Clinical and experimental studies of benign prostatic hyperplasia," *Urol Clin North Am* 17:461, 1990 (canine); Brendler CB *et al.*, "Spontaneous benign prostatic hyperplasia in the beagle," *J Clin Invest* (1983) 71:1114, 1983; Winter ML *et al.*, "Induction of benign prostatic hyperplasia in intact dogs by near-physiological levels of 5-alpha-dihydrotestosterone and 17-beta-estradiol," *Prostate* 26: 325, 1995; Banerjee PP *et al.*, "Age-dependent and lobe-specific spontaneous hyperplasia in the Brown Norway rat prostate," *Biol Reprod* 59: 1163, 1998; Van Coppenolle F *et al.*, "Pharmacological effects of the lipidosterolic extract of Serenoa repens (Permixon) on rat prostate hyperplasia induced by hyperprolactinemia: comparison with finasteride," *Prostate* 43:49, 2000; Steiner MS *et al.*, "The chimpanzee as a model of human benign prostatic hyperplasia," *J Urol* 162:1454, 1999; and Shishido

T *et al.*, *Nippon Hinyokika Gakkai Zasshi* 91:459, 2000 (guinea pig).

Alternatively, cellular extracts may be prepared from normal human prostate tissue or available cell lines, particularly prostate derived cell lines.

A variety of methods can be used to obtain an extract of a cell. Cells can be disrupted using either physical or chemical disruption methods. Examples of physical disruption methods include, but are not limited to, sonication and mechanical shearing. Examples of chemical lysis methods include, but are not limited to, detergent lysis and enzyme lysis. Extracts may be further manipulated before use in the methods of the invention, for example by fractionation or by other types of purification. A skilled artisan can readily adapt methods for preparing cellular extracts in order to obtain extracts for use in the present methods.

Once an extract of a cell is prepared, the extract is mixed with the Del-1 protein or a variant thereof under conditions in which association of the protein with the binding partner can occur. A variety of conditions can be used, the most preferred being conditions that closely resemble conditions found in the cytoplasm of a human cell. Features such as osmolarity, pH, temperature, and the concentration of cellular extract used, can be varied to optimize the association of the protein with the binding partner.

After mixing under appropriate conditions, the bound complex is separated from the mixture. A variety of techniques can be utilized to separate the mixture. For example, antibodies specific to a Del-1 protein or a variant thereof can be used to immunoprecipitate the binding partner complex. Alternatively, standard chemical separation techniques such as chromatography and density/sediment centrifugation can be used.

After removal of non-associated cellular constituents found in the extract, the binding partner can be dissociated from the complex using conventional methods. For example, dissociation can be accomplished by altering the salt concentration or pH of the mixture.

To aid in separating associated binding partner pairs from the mixed extract, the Del-1 protein or a variant thereof can be immobilized on a solid support. For example, the protein can be attached to a nitrocellulose matrix or acrylic beads. Attachment of the protein to a solid support aids in separating peptide/binding partner pairs from other constituents found in the extract. The identified binding partners can be either a single protein or a complex made up of two or more proteins. Alternatively, binding partners may be identified using a Far-Western assay according to the procedures of Takayama *et*

al., *Methods Mol Biol* 69:171-184, 1997 or Sauder *et al.*, *J GenVirol* 77:991-996, 1996, or identified through the use of epitope tagged proteins or GST fusion proteins.

Alternatively, the nucleic acid molecules encoding a Del-1 protein or a variant thereof can be used in a yeast two-hybrid system. The yeast two-hybrid system has been used to identify other protein partner pairs and can readily be adapted to employ the nucleic acid molecules herein described.

D. Methods to Identify Agents that Modulate the Expression a Nucleic Acid Encoding the Proteins Associated with BPH

Another embodiment of the present invention provides methods for identifying agents that modulate the expression of a nucleic acid encoding a Del-1 protein, such as a protein having the amino acid sequence of SEQ ID NO: 2, or a variant thereof. Such assays may utilize any available means of monitoring for changes in the expression level of the nucleic acids encoding Del-1 protein or a variant thereof. As used herein, an agent is said to modulate the expression of a nucleic acid encoding the Del-1 protein or a variant thereof, if it is capable of up- or down-regulating expression of the nucleic acid in a cell.

In one assay format, cell lines associated with BPH, that contain reporter gene fusions between part or all of the open reading frame defined by nucleotides 406-1845 of SEQ ID NO: 1, and/or the 5′ and/or 3′ regulatory sequences and any assayable fusion partner may be prepared. Numerous assayable fusion partners are known and readily available including the firefly luciferase gene and the gene encoding chloramphenicol acetyltransferase (Alam *et al.*, *Anal Biochem* 188:245-254, 1990). Cell lines containing the reporter gene fusions are then exposed to the agent to be tested under appropriate conditions and time. Differential expression of the reporter gene between samples exposed to the agent and control samples identifies agents which modulate the expression of a nucleic acid encoding the Del-1 protein, such as a protein having the amino acid sequence of SEQ ID NO: 2, or a variant thereof.

Additional assay formats may be used to monitor the ability of the agent to modulate the expression of a nucleic acid encoding a Del-1 protein of the invention, such as the protein having the amino acid sequence of SEQ ID NO: 2, or a variant thereof. For instance, mRNA expression may be monitored directly by hybridization to the nucleic acids encoding the Del-1 protein or a variant thereof. Prostate cell lines or tissues or cell lines associated with BPH are exposed to the agent to be tested under appropriate

conditions and time and total RNA or mRNA is isolated by standard procedures such those disclosed in Sambrook *et al.*, (*supra*).

Probes to detect differences in RNA expression levels between cells exposed to the agent and control cells may be prepared from the nucleic acids encoding a Del-1 protein, such as a protein having the amino acid sequence of SEQ ID NO: 2, or a variant thereof. It is preferable, but not necessary, to design probes which hybridize only with target nucleic acids under conditions of high stringency. Only highly complementary nucleic acid hybrids form under conditions of high stringency. Accordingly, the stringency of the assay conditions determines the amount of complementarity which should exist between two nucleic acid strands in order to form a hybrid. Stringency should be chosen to maximize the difference in stability between the probe:target hybrid and probe:non-target hybrids.

Probes may be designed from the nucleic acids encoding a Del-1 protein or a variant thereof through methods known in the art. For instance, the G+C content of the probe and the probe length can affect probe binding to its target sequence. Methods to optimize probe specificity are commonly available in Sambrook *et al.*, (*supra*) or Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Co., 1995.

Hybridization conditions are modified using known methods, such as those described by Sambrook *et al.* and Ausubel *et al.* as required for each probe. Hybridization of total cellular RNA or RNA enriched for polyA RNA can be accomplished in any available format. For instance, total cellular RNA or RNA enriched for polyA RNA can be affixed to a solid support and the solid support exposed to at least one probe comprising at least one, or part of one, of the sequences of Del-1 protein or a variant thereof under conditions in which the probe will specifically hybridize. Alternatively, nucleic acid fragments comprising at least one, or part of one, of the sequences of Del-1 protein or a variant thereof can be affixed to a solid support, such as a silicon chip or a porous glass chip or wafer. The wafer can then be exposed to total cellular RNA or polyA RNA from a sample under conditions in which the affixed sequences will specifically hybridize. Such solid supports and hybridization methods are widely available, for example, those disclosed by Beattie, WO 95/11755 (1995). By examining for the ability of a given probe to specifically hybridize to an RNA sample from an untreated cell population and from a cell population exposed to the agent, agents which up or down regulate the expression of a nucleic acid encoding the protein having the sequence of SEQ ID NO: 2 are identified.

Hybridization for qualitative and quantitative analysis of mRNAs may also be carried out by using a RNase Protection Assay (*i.e.*, RPA, see Ma *et al.*, *Methods* 10: 273-238, 1996). Briefly, an expression vehicle comprising cDNA encoding the gene product and a phage specific DNA dependent RNA polymerase promoter (*e.g.*, T7, T3 or SP6 RNA polymerase) is linearized at the 3' end of the cDNA molecule, downstream from the phage promoter, wherein such a linearized molecule is subsequently used as a template for synthesis of a labeled antisense transcript of the cDNA by *in vitro* transcription. The labeled transcript is then hybridized to a mixture of RNA isolated from cells associated with BPH (*i.e.*, total or fractionated mRNA), by incubation at 45°C overnight in a buffer comprising 80% formamide, 40 mM Pipes, pH 6.4, 0.4 M NaCl and 1 mM EDTA. The resulting hybrids are then digested in a buffer comprising 40 μg/ml ribonuclease A and 2 μg/ml ribonuclease. After deactivation and extraction of extraneous proteins, the samples are loaded onto urea/polyacrylamide gels for analysis.

In another assay to identify agents which effect the expression of the instant gene products, cells or cell lines associated with BPH are first identified which physiologically express Del-1 protein or a variant thereof (*e.g.*, by using assays of tissue distribution via Northern blot, although RPAs may serve the identical purpose of expression selection). Cells and/or cell lines so identified would be expected to comprise the necessary cellular machinery such that the fidelity of modulation of the transcriptional apparatus is maintained with regard to exogenous contact of agent with appropriate surface transduction mechanisms and/or the cytosolic cascades. Further, such cells or cell lines are transduced or transfected with an expression vehicle (*e.g.*, a plasmid or viral vector) construct comprising an operable non-translated 5'-promoter-containing end of the structural gene encoding the instant gene products fused to one or more antigenic fragments, which are peculiar to the Del-1 protein or variant, wherein said fragments are under the transcriptional control of said promoter and are expressed as polypeptides whose molecular weight can be distinguished from the naturally occurring polypeptides or may further comprise an immunologically distinct tag or other detectable marker. Such a process is well known in the art (see Sambrook et al., *supra*).

The prostate cell lines or tissues, or cells or cell lines associated with BPH, transduced or transfected as outlined above, are then contacted with agents under appropriate conditions. For example, the agent comprises a pharmaceutically acceptable excipient and is contacted with cells comprised in an aqueous physiological buffer such as phosphate buffered saline (PBS) at physiological pH, Eagles balanced salt solution

(BSS) at physiological pH, PBS or BSS comprising serum or conditioned media comprising PBS or BSS and/or serum incubated at 37°C. Said conditions may be modulated as deemed necessary by one of skill in the art. Subsequent to contacting the cells with the agent, said cells are disrupted and the polypeptides of the lysate are fractionated such that a polypeptide fraction is pooled and contacted with an antibody to be further processed by immunological assay (*e.g.*, ELISA, immunoprecipitation or Western blot). The pool of proteins isolated from the "agent-contacted" sample is then compared with a control sample where only the excipient is contacted with the cells, and an increase or decrease in the immunologically generated signal from the "agent-contacted" sample compared to the control is used to distinguish the effectiveness of the agent.

E. Methods to Identify Agents that Modulate the Levels of or at Least One Activity of the BPH-Associated Proteins

Another embodiment of the present invention provides methods for identifying agents that modulate at least one activity of a Del-1 protein, such as the protein having the amino acid sequence of SEQ ID NO: 2, or a variant thereof. Such methods or assays utilize cells associated with BPH, and may utilize any means of monitoring or detecting the desired activity. For example, the method or assay could measure an activity such as binding to or clustering of endothelial cell receptors of the integrin family such as the $\alpha v\beta 3$ receptor, the formation of focal complexes or recruitment of talin and vinculin into these complexes, the phosphorylation of proteins within the focal complexes (e.g. of $p125^{FAK}$, MAPK or Shc), endothelial cell adhesion or migration, angiogenesis or vascular formation (Penta K *et al.*, *J Biol Chem* 274:11101-11109, 1999).

In one format, the relative amounts of a Del-1 protein, or a variant thereof, between a cell population that has been exposed to the agent to be tested compared to an un-exposed control cell population may be assayed. In this format, probes such as specific antibodies are used to monitor the differential expression of the protein in the different cell populations. Cell lines or populations are exposed to the agent to be tested under appropriate conditions and time. Cellular lysates may be prepared from the exposed cell line or population and a control, unexposed cell line or population. The cellular lysates are then analyzed with the probe.

Antibody probes are prepared by immunizing suitable mammalian hosts in appropriate immunization protocols using a sequence of six or more consecutive amino

acids from the Del-1 protein or a variant thereof, or if desired, or if required, to enhance immunogenicity, the selected peptide sequence may be conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co. (Rockford, IL), may be desirable to provide accessibility to the hapten. The hapten peptides can be extended at either the amino or carboxy terminus with a cysteine residue or interspersed with cysteine residues, for example, to facilitate linking to a carrier. Administration of the immunogens is conducted generally by injection over a suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers of antibodies are taken to determine adequacy of antibody formation.

While the polyclonal antisera produced in this way may be satisfactory for some applications, the use of monoclonal preparations is preferred for pharmaceutical compositions. Immortalized cell lines which secrete the desired monoclonal antibodies may be prepared using the standard method of Kohler and Milstein (*Nature* 256:495-497, 1975) or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the peptide hapten, polypeptide or protein. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either *in vitro* or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab', or $F(ab')_2$ fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Antibody regions that bind specifically to the desired regions of the protein can also be produced in the context of chimeras with multiple species origin, such as humanized antibodies.

Agents that are assayed in the above method can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected

when the agent is chosen randomly without considering the specific sequences involved in the association of a Del-1 protein (or a variant thereof) alone or with its associated substrates, binding partners, etc. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library, or a growth broth of an organism.

As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a nonrandom basis which takes into account the sequence of the target site and/or its conformation in connection with the agent's action. As described in the Examples, there are proposed binding motifs, hydroxylation and other consensus sites in the protein having SEQ ID NO: 2. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up these sites. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to an EGF-like domain or hydroxylation site in SEQ ID NO: 2, as described in the Examples.

The agents assayed using the method of the present invention can be, as examples, peptides, small molecules, vitamin derivatives, as well as carbohydrates. Dominant negative proteins, DNAs encoding these proteins, antibodies to these proteins, peptide fragments of these proteins or mimics of these proteins may be introduced into cells to affect function. "Mimic" used herein refers to the modification of a region or several regions of a peptide molecule to provide a structure chemically different from the parent peptide but topographically and functionally similar to the parent peptide (see Grant GA. in: Meyers (ed.) Molecular Biology and Biotechnology, pp. 659-664, VCH Publishers, New York, 1995). A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents that can be assayed in the methods of the present invention.

The peptide agents assayed in the methods of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Another class of agents that may be assayed in the methods of the present invention are antibodies immunoreactive with critical positions within a Del-1 protein or variant thereof. Antibody agents are obtained by immunization of suitable mammalian

subjects with peptides, containing as antigenic regions, those portions of the protein intended to be targeted by the antibodies.

F. Uses for Agents that Modulate Expression or at Least One Activity of the BPH-Associated Proteins

Another embodiment of the present invention provides methods for modulating the expression or at least one activity of a Del-1 protein or a variant thereof, in a subject with benign prostatic hyperplasia. As provided in the Examples, the nucleic acid encoding the Del-1 protein having the amino acid sequence of SEQ ID NO: 2 is differentially expressed in BPH tissue compared to normal tissue. Agents that modulate or up- or down-regulate the expression of the Del-1 protein (or a variant thereof) or agents, such as agonists or antagonists that modulate at least one activity of the protein (or a variant thereof), may be used to modulate biological and pathologic processes associated with the protein's function and activity in a subject with BPH.

As used herein, a subject can be any mammal. The term "mammal" is defined as an individual belonging to the class Mammalia. The agent is particularly useful in the treatment of human subjects.

Pathological processes refer to a category of biological processes which produce a deleterious effect in BPH. For example, expression of a Del-1 protein (or a variant thereof) may be associated with prostate cell growth or hyperplasia. As used herein, an agent is said to modulate a pathological process when the agent reduces the degree or severity of the process. For instance, BPH may be prevented or disease progression modulated by the administration of agents which up- or down-regulate or modulate in some way the expression or at least one activity of a Del-1 protein or variant thereof.

Modulatory agents used in the methods of the present invention can be provided alone, or in combination with other agents that modulate a particular pathological process. For example, an agent of the present invention can be administered in combination with other known drugs. As used herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a fashion such that the agents will act at the same time.

The agents used in methods of the present invention can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the

recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The present invention further provides methods using compositions containing one or more agents which modulate expression or at least one activity of a Del-1 protein or a variant thereof, in a subject with benign prostatic hyperplasia. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typical dosages comprise 0.1 to 100 μg/kg body wt. The preferred dosages comprise 0.1 to 10 μg/kg body wt. The most preferred dosages comprise 0.1 to 1 μg/kg body wt.

In addition to the pharmacologically active agent, the compositions used in the methods of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, *e.g.*, sesame oil, or synthetic fatty acid esters, *e.g.*, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, and the substances may include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be coadministered along with other compounds typically prescribed for these conditions

according to generally accepted medical practice. The compounds of this invention can be utilized *in vivo*, ordinarily in mammals, such as humans, sheep, horses, cattle, pigs, dogs, cats, rats and mice, or *in vitro*.

G. Diagnostic Methods

As the gene for Del-1 is differentially expressed in BPH tissue compared to normal prostate tissue, the level of expression of Del-1 protein or variants thereof, and of the genes that encode these proteins, may be used to diagnose or monitor BPH, prostate function, or to track disease progression.

One means of diagnosing BPH using the nucleic acid molecules encoding Del-1 protein (or variants thereof) or protein molecules themselves involves obtaining prostate tissue from living subjects. Obtaining tissue samples from living sources is problematic for tissues such as prostate. However, due to the nature of the treatment paradigms for BPH, biopsy may be necessary. Methods of the invention may involve treatment of tissues with collagenases or other proteases to make the tissue amenable to cell lysis (Semenov *et al.*, *Biull Eksp Biol Med* 104, 113-116, 1987). Further, it is possible to obtain biopsy samples from different regions of the prostate for analysis. When possible, urine, blood, peripheral lymphocytes or other tissue samples may be used as the tissue sample in the assay.

The use of molecular biological tools has become routine in forensic technology. For example, nucleic acid probes may be used to determine the expression of a nucleic acid molecule comprising all or at least part of the sequence of Del-1 (SEQ ID NO: 1) or variants thereof, as described above, in forensic/pathology specimens. Further, nucleic acid assays may be carried out by any means of conducting a transcriptional profiling analysis. In addition to nucleic acid analysis, forensic methods of the invention may target a Del-1 protein, such as the protein comprising SEQ ID NO: 2, or variants described above, to determine up- or down-regulation of the genes (Shiverick *et al.*, *Biochim Biophys Acta* 393:124-133, 1975).

Assays to detect nucleic acid or protein molecules of the invention may be in any available format. Typical assays for nucleic acid molecules include hybridization or PCR based formats. In one assay format, mRNA expression may be monitored directly by hybridization to the nucleic acids probes. Total RNA or mRNA is isolated from patient samples by standard procedures such those disclosed in Sambrook et al., (*supra*). See section D for a discussion of commonly available assay formats and Example 3 for

an example of a PCR based assay. In preferred embodiments, assays are carried-out with appropriate controls.

Typical assays for the detection of a Del-1 protein or variant thereof include the use of antibody probes in any available format such as *in situ* binding assays, etc. (see Harlow & Lane, Antibodies - A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988. For example, in *in vitro* assays, cells from test samples are disrupted and the polypeptides of the lysate are fractionated such that a polypeptide fraction is pooled and contacted with an antibody to be further processed by immunological assay (e.g., ELISA, immunoprecipitation or Western blot). The pool of proteins isolated from the test sample will be compared with a control sample and an increase or decrease in the immunologically generated signal from the test sample compared to the control will be used to distinguish the effectiveness of the agent.

The above methods may also be used in other diagnostic protocols, including protocols and methods to detect disease states in other tissues or organs, for example the tissues in which gene expression is detected (see Figure 2 and Figure 3).

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, practice the claimed methods of the present invention. The following working examples, therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLES

Example 1

Identification of Differentially Expressed BPH mRNA

Human tissue was obtained from the transitional zone of the prostate in biopsy samples from normal individuals and from patients with BPH. BPH was defined histologically in all samples. Normal tissue and asymptomatic BPH samples came from individuals who died of trauma, and did not report symptoms. Patients having BPH with symptoms were defined as those with a need for frequent urination; in these patients a radical prostatectomy had been performed. Prostate cancer patients provided age-matched tissue samples for symptomatic BPH patients, but were without symptoms and without cancer in the transitional zone under examination.

Microarray sample preparation was conducted with minor modifications,

following the protocols set forth in the Affymetrix GeneChip® Expression Analysis Manual. Frozen tissue was ground to a powder using a Spex Certiprep 6800 Freezer Mill. Total RNA was extracted with Trizol® (GibcoBRL) utilizing the manufacturer's protocol. The total RNA yield for each sample was 200-500 g per 300 mg tissue weight. mRNA was isolated using the Oligotex mRNA Midi kit® (Qiagen) followed by ethanol precipitation. Double stranded cDNA was generated from mRNA using the SuperScript Choice® system (GibcoBRL). First strand cDNA synthesis was primed with a T7-(dT24) oligonucleotide. The cDNA was phenol-chloroform extracted and ethanol precipitated to a final concentration of 1 g/l. From 2 g of cDNA, cRNA was synthesized using Ambion's T7 MegaScript in vitro Transcription Kit®.

To biotin label the cRNA, nucleotides Bio-11-CTP and Bio-16-UTP (Enzo Diagnostics) were added into the reaction. Following a 37C incubation for six hours, impurities were removed from the labeled cRNA following the RNAeasy Mini kit protocol (Qiagen). cRNA was fragmented (5' fragmentation buffer consisting of 200 mM Tris-acetate (pH 8.1), 500 mM KOAc, 150 mM MgOAc) for thirty-five minutes at 94C. Following the Affymetrix protocol, 55 g of fragmented cRNA was hybridized on the Human Hu35KsubA gene chip set and the HuGeneFL array for twenty-four hours at 60 rpm in a 45C hybridization oven. The chips were washed and stained with Streptavidin Phycoerythrin (SAPE) (Molecular Probes) in Affymetrix fluidics stations. To amplify staining, SAPE solution was added twice with an anti-streptavidin biotinylated antibody (Vector Laboratories) staining step in between. Hybridization to the probe arrays was detected by fluorometric scanning (Hewlett Packard Gene Array Scanner). Data was analyzed using Affymetrix GeneChip version 3.0 and Expression Data Mining Tool (EDMT) software (version 1.0).

Differential expression of genes between the BPH and normal prostate samples was determined using the Affymetrix Hu35KsubA GeneChip set by the following criteria: (1) For each gene, Affymetrix GeneChip average difference values were determined by standard Affymetrix EDMT software algorithms, which also made "Absent" (=not detected), "Present" (=detected) or "Marginal" (=not clearly Absent or Present) calls for each GeneChip element; (2) all negative values (=Absent) were raised to a floor of +20 (positive 20) so that fold change calculations could be made where values were not already greater than or equal to +20; (3) median levels of expression were compared between the normal control group and the BPH with symptoms disease group to obtain greater than or equal 3-fold up/down values; (4) The median value for

the higher expressing group needed to be greater or equal to 200 average difference units in order to be considered for statistical significance; (5) Genes passing the criteria of #1-4 were analyzed for statistical significance using a two-tailed T test and deemed statistically significant if $p < 0.05$.

AA256268 exhibited a 4.2 fold change in expression levels in tissue from BPH patients with symptoms compared to normal prostate tissue ($p=0.0015$).

Example 2

Cloning of a Full Length Human cDNA Corresponding to the Differentially Expressed mRNA Species

The full length human cDNA (SEQ ID NO: 1) corresponding to the differentially expressed mRNA species identified was obtained. Briefly, a pair of gene specific primers were synthesized and a fetal brain cDNA library was screened. Of a number of positive clones obtained, the longest one that corresponded well to the predicted size of the RNA transcript, as determined by Northern blotting, was selected for DNA sequencing.

The nucleotide sequences of the full-length human cDNA corresponding to the differentially regulated mRNAs detected above is set forth in SEQ ID NO: 1. The cDNA comprises 4690 base pairs with an open reading frame at nucleotides 406-1845 encoding a protein of 480 amino acids. The corresponding amino acid sequence for the encoded protein is set forth in SEQ ID NO: 2.

Analysis of the amino acid sequence of SEQ ID NO: 2 predicts EGF-like domains starting at amino acids 48, 105 and 143; a calcium binding EGF-like domain starting at amino acids 119; an ATP/GTP-binding site motif starting at amino acid 249; and an aspartic acid and asparagine hydroxylation site starting at amino acid 134. A hydrophobicity plot (PEPPLOT) of the amino acid sequence for AA256268 is shown in Figure 5.

A Blast of this sequence against the GenBank database indicated that this protein AA256268 had 100% identity with an integrin-binding protein known as Del-1 (GenBank accession number U70312).

Figure 1 shows the expression of AA256268 across 31 human samples, including 10 normals, 5 BPH samples from individuals without symptoms, 8 BPH samples from individuals without symptoms who were prostate cancer patients, and 8 BPH samples from patients with symptoms. Upregulation of expression is observed in BPH samples

from people with symptoms as well as in BPH samples from cancer patients. The expression data show that up-regulation of AA256268 is diagnostic for BPH in patients with symptoms.

Figure 2 shows relative AA256268 mRNA levels determined via Northern Blot in a range of normal human tissues. A probe based on SEQ ID NO: 1 (probe F156/R759) was exposed to human mRNA blots (available from ClonTech) overnight at 65C in Church-Gilbert hybridization buffer, following standard methodology as described by Sambrook et al. (1989).

Figure 3 shows an electronic Northern, in which the expression level was measured across a panel of human normal and BPH tissues using the Affymetrix Hu35KsubA GeneChip set. For each tissue type, the mean +/- SDM is shown as a horizontal bar graph for samples obtained from 3 or more normal individuals. The order of expression (high to low) across the normal tissues in Figure 3 is: brain >lung >bone =myometrium >cervix =colon =rectum >small intestine =stomach >endometrium >muscles >ovary >>esophagous >kidney =skin =liver =breast >tonsil >spleen >thymus >>blood. Thus, based upon the tissue panel tested, expression in normal tissue is quite strongly restricted. Because BPH with symptoms is associated with hypertrophic growth of the prostate and/or a low grade chronic inflammation, upregulated expression of AA256268 is likely of diagnostic value in other tissues for disease states involving hypertrophic growth and/or inflammation.

Example 3

Quantitative PCR Analysis of Expression Levels

Figure 4 shows the results of the semi-quantitative PCR analysis of expression levels of mRNA corresponding to SEQ ID NO: 1 in various human tissue samples. Real time PCR detection was accomplished by the use of the ABI PRISM 7700 Sequence Detection System. The 7700 measures the fluorescence intensity of the sample each cycle and is able to detect the presence of specific amplicons within the PCR reaction. Each sample was assayed for the level of GAPDH and mRNA corresponding to SEQ ID NO: 1. GAPDH detection was performed using Perkin Elmer part no. 402869 according to the manufacturer's directions. Primers were designed from SEQ ID NO: 1 using Primer Express®, a program developed by PE, to efficiently find primers and probes for specific sequences. These primers were used in conjunction with SYBR® green (Molecular Probes), a nonspecific double stranded DNA dye, to measure the expression

level mRNA corresponding to SEQ ID NO: 1, which was normalized to the GAPDH level in each sample.

The methods of the invention can be used to distinguish among various types of prostate disease diagnostically and prognostically, or to track disease progress. As shown by the GeneChip® data for the extended patient panel (Figure 1) and the electronic Northern (Figure 3), the nucleic acid sequences corresponding to SEQ ID NO: 1 (DNA and mRNA) are differentially regulated in various prostate diseases, such as BPH. In addition, the nucleic acid sequences corresponding to SEQ ID NO: 1 are differentially expressed among various BPH states compared to normal prostate tissue, such as in patients that have cancer, patients that are symptomatic, and patients that are not symptomatic. Therefore, measuring the total amount of transcript as well as the relative expression level of the gene may be used to stratify patients diagnostically and prognostically.

Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims. All cited patents, patent applications and publications referred to in this application are herein incorporated by reference in their entirety.

What Is Claimed:

1. A method of diagnosing benign prostatic hyperplasia in a patient, comprising determining in a patient sample the level of expression of nucleic acid molecule encoding a Del-1 protein or a variant thereof.

2. The method of claim 1, wherein the Del-1 protein or the variant thereof is encoded by (a) an isolated nucleic acid molecule that encodes the amino acid sequence of SEQ ID NO: 2; or (b) an isolated nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule that encodes the amino acid sequence of SEQ ID NO: 2.

3. The method of claim 1 or 2, wherein the patient does not exhibit the symptoms of benign prostatic hyperplasia.

4. A method of identifying binding partners for a Del-1 protein or a variant thereof, comprising:

exposing said protein to a potential binding partner derived from prostate cells or tissue; and

determining if the potential binding partner binds to said protein, thereby identifying binding partners for the Del-1 protein or the variant thereof.

5. The method of claim 4 wherein the potential binding partner is in a prostate cell extract.

6. The method of claim 5 wherein the cells are obtained from human biopsy samples.

7. The method of claim 4 or 5 wherein the cells are obtained from an animal model of prostate disease.

8. A method of identifying an agent which modulates the expression of a nucleic acid encoding a Del-1 protein or a variant thereof, comprising:

exposing prostate cells to the agent; and

determining whether the agent modulates expression of said nucleic acid, thereby identifying an agent which modulates the expression of a nucleic acid encoding the Del-1 protein or the variant thereof.

9. The method of claim 8 wherein the cells are in vitro.

10. The method of claim 8 wherein the cells are obtained from human prostate biopsy tissue.

11. The method of claim 8 wherein the cells are obtained from an animal model of prostate disease.

12. A method of identifying an agent which modulates at least one activity of a Del-1 protein or a variant thereof, comprising:

exposing prostate cells to the agent; and

determining whether the agent modulates at least one activity of said protein, thereby identifying an agent which modulates at least one activity of the Del-1 protein or the variant thereof.

13. The method of claim 12 wherein the cells are in vitro.

14. The method of claim 12 wherein the cells are obtained from human prostate biopsy tissue.

15. The method of claim 12 wherein the cells are obtained from an animal model of prostate disease.

16. The method of any of claims 12-15 wherein said activity is selected from the group consisting of binding to or clustering of endothelial cell receptors of the integrin family, formation of focal complexes, recruitment of talin and vinculin into focal complexes, phosphorylation of proteins within the focal complexes endothelial cell adhesion, endothelial cell migration, angiogenesis and vascular formation.

17. The method of claim 16 wherein said activity is the binding to or clustering of

endothelial cell receptors of the integrin family.

18. The method of claim 17 [wherein said endothelial cell receptors of the integrin family are αβv3 receptors.

19. The method of claim 16 wherein said activity is the phosphorylation of $p125^{FAK}$, MAPK or Shc.

20. A method of modulating the expression of a nucleic acid encoding a Del-1 protein or a variant thereof, in a subject with benign prostatic hyperplasia, comprising:

administering to the subject an effective amount of an agent which modulates the expression of a nucleic acid encoding the Del-1 protein or the variant thereof.

21. A method of modulating at least one activity of a Del-1 protein or a variant thereof, in a subject with benign prostatic hyperplasia, comprising:

administering to the subject an effective amount of an agent which modulates at least one activity of the Del-1 protein or the variant thereof.

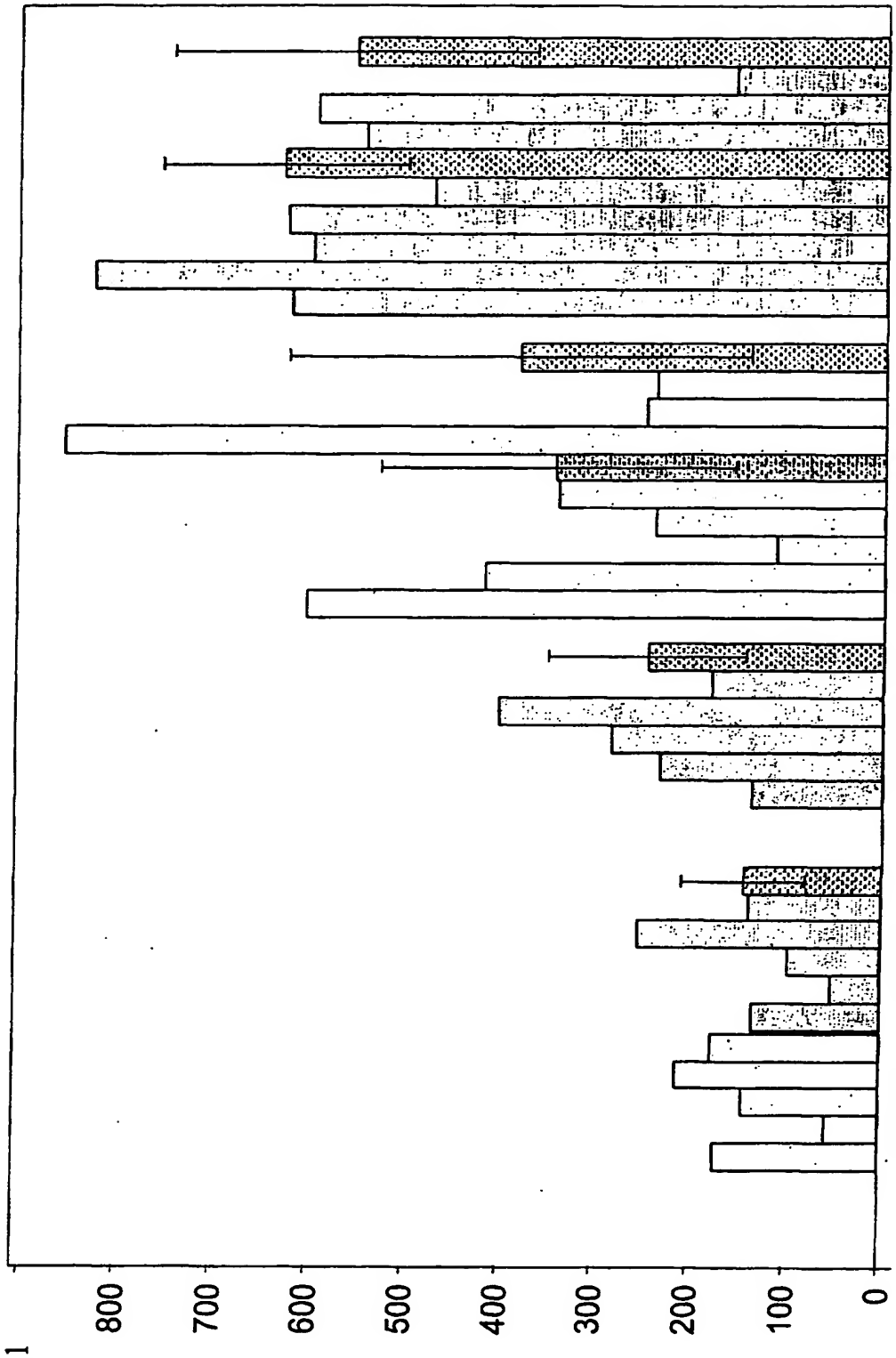

Fig. 1

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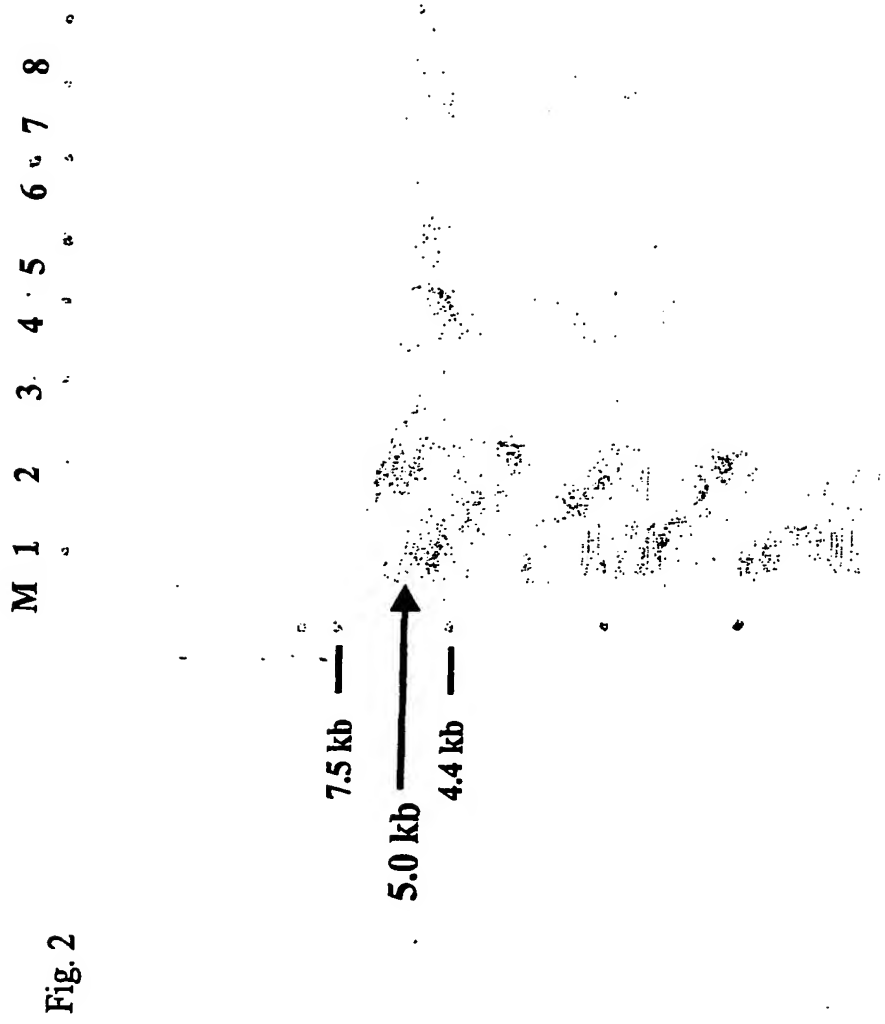

Fig. 2

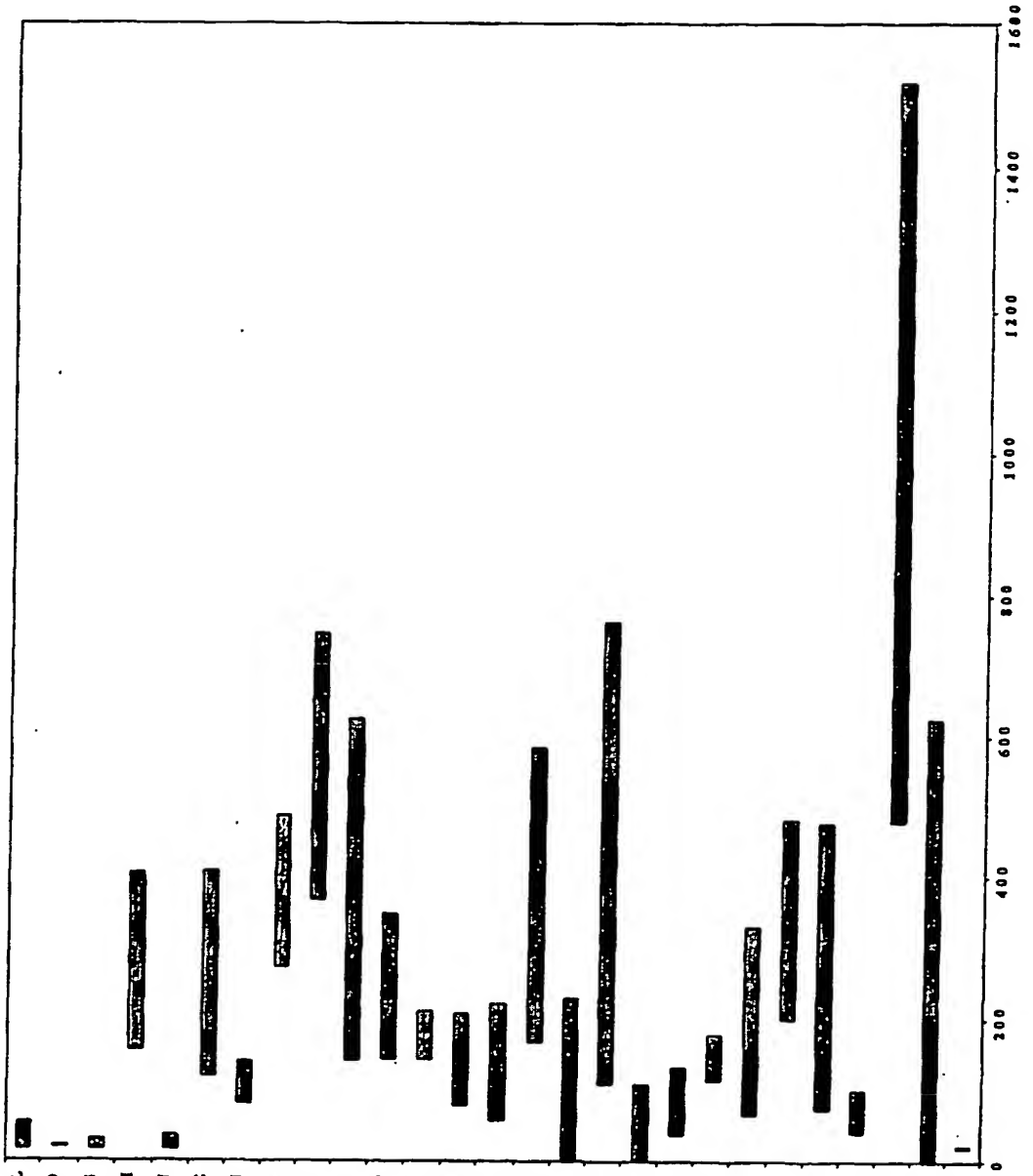

Fig. 3

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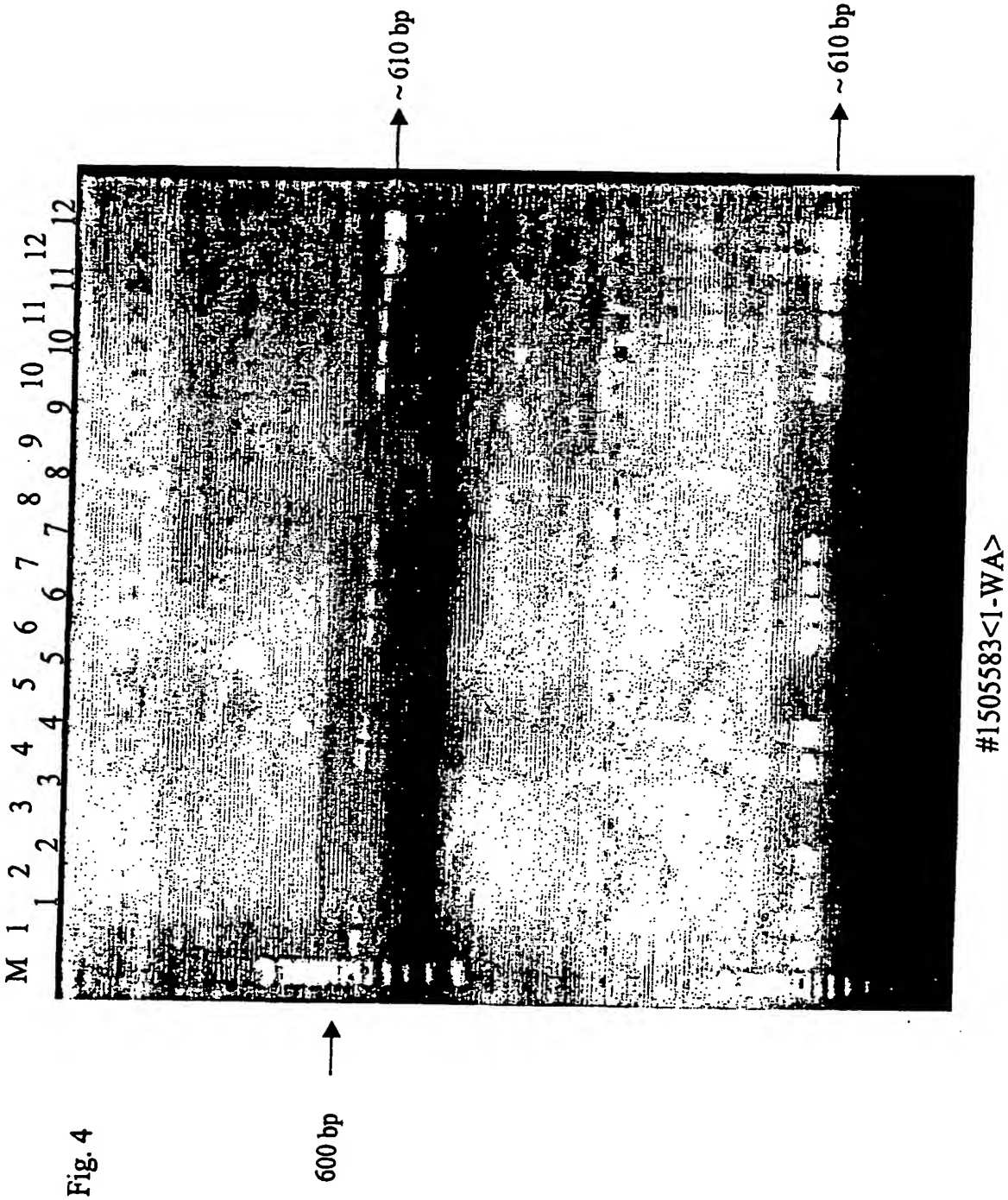

Fig. 4

Sequence Listing

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Kulkarni, Prakash
Getzenberg, Robert H.

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